PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 31/33, C07D 498/18
C07D 498/22 // (C07D 498/18
C07D 311/00, 273/00, 221/00)
(C07D 498/22, 311/00, 311/00
C07D 273/00, 221/00)

A1

(11) International Publication Number: WO 91/04025

(43) International Publication Date: 4 April 1991 (04.04.91)

(21) International Application Number: PCT/GB90/01412

(22) International Filing Date: 13 September 1990 (13.09.90)

(30) Priority data:

8920849.0	14 September 1989 (14.09.89)	GB
8920985.2	15 September 1989 (15.09.89)	GB
9006449.4	22 March 1990 (22.03.90)	GB
9012795.2	8 June 1990 (08.06.90)	GB
9014959.2	6 July 1990 (06.07.90)	GB

(71) Applicant *(for all designated States except US):* FISONS PLC [GB/GB]; Fison House, Princes Street, Ipswich, Suffolk IP1 1QH (GB).

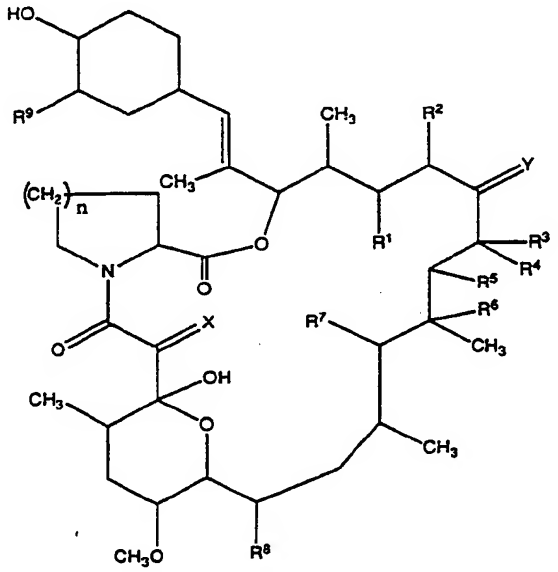
(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : DONALD, David, Keith [GB/GB]; Orchardside, 50 Avenue Road, Ashby de la Zouch, Leicestershire LE6 5FE (GB). FURBER, Mark [GB/GB]; 24 Derby Road, Kegworth, Derby DE7 2EN (GB). HARDERN, David, Norman [GB/GB]; 6 Charnwood Fields, Sutton Bonington, Loughborough, Leicestershire LE12 5NP (GB). LEFF, Paul [GB/GB]; 3 Kegworth Lane, Long Whatton, Leicestershire LE12 5BB (GB).

(74) Agent: JONES, Stephen, Anthony; Fisons plc, 12 Derby Road, Loughborough, Leicestershire LE11 OBB (GB).

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: NOVEL MACROCYCLIC COMPOUNDS AND NOVEL METHOD OF TREATMENT



(I)

(57) Abstract

Compounds of formula (I), wherein R^1 and R^2 independently represent H or OH, or a second carbon-carbon bond; R^3 represents optionally substituted methyl, ethyl or propyl; R^4 represents H; R^5 and R^6 represent a second carbon-carbon bond; R^7 represents H or OH; R^8 represents OCH_3; R^9 represents OH or OCH_3; X and Y independently represent O or (H, OH); n represents 1 or 2; and in addition some of the substituents form rings with each other; with various provisos; are indicated in the treatment of immunodepression. A number of novel compounds of formula (I) are also provided.

P.11+4

* See back of page

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MC	Monaco
AU	Australia	FI	Finland	MG	Madagascar
BB	Barbados	FR	France	ML	Mali
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Fasso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GR	Greece	NL	Netherlands
BJ	Benin	HU	Hungary	NO	Norway
BR	Brazil	IT	Italy	PL	Poland
CA	Canada	JP	Japan	RO	Romania
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CM	Cameroon	LI	Liechtenstein	SU	Soviet Union
DE	Germany	LK	Sri Lanka	TD	Chad
DK	Denmark	LU	Luxembourg	TG	Togo
				US	United States of America

NOVEL MACROCYCLIC COMPOUNDS AND NOVEL METHOD OF TREATMENT

This invention relates to novel pharmaceutical uses of certain known macrocyclic compounds, and to novel macrocyclic compounds which have the same novel utility.

European patent application No 184162 (to Fujisawa Pharmaceuticals Co Ltd) discloses several macrocyclic compounds which are derivatives of 12-(2-cyclohexyl-1-methylvinyl)-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene (numbered FR-900506, FR-900520, FR-900523 and FR-900525) and which are isolated from microorganisms belonging to the genus Streptomyces. The macrocyclic compounds and a number of their derivatives are indicated as immunosuppressive agents.

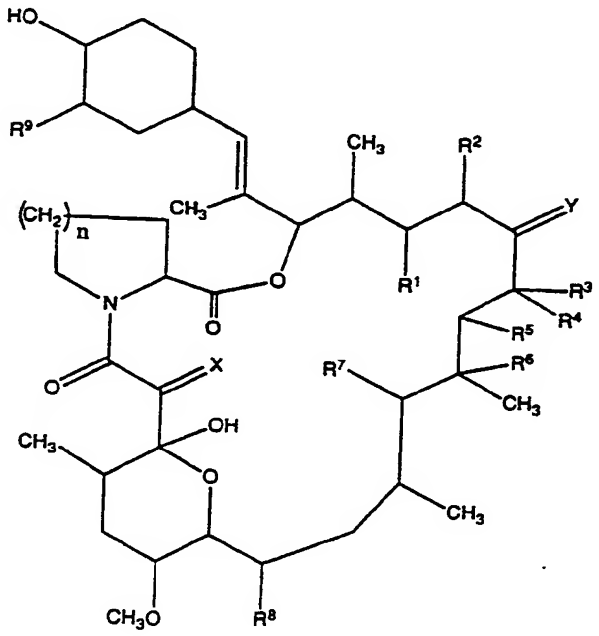
International patent application No WO 89/05304 (also European Patent application No 323042, both to Fisons plc) discloses a large number of macrocyclic compounds which may be derived from those disclosed in European patent application No 184162. Again, the compounds are primarily indicated as immunosuppressive agents.

European patent applications Nos 349049 and 349061 (both to Merck & Co Inc) each disclose a macrocyclic compound which is indicated as an immunosuppressive agent.

The compounds and methods disclosed in the patent applications mentioned above may be used in the production of the novel compounds of the present invention. Alternatively, the novel compounds may be produced by total synthesis.

We have now found a novel group of macrocyclic compounds which act as antagonists of immunosuppressive compounds, particularly macrocyclic immunosuppressive compounds including derivatives of 12-(2-cyclohexyl-1-methylvinyl)-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene and rapamycin, as shown in the mixed lymphocyte reaction (MLR) (described in WO 89/05304, Example A). The novel group of compounds are therefore useful inter alia in the treatment of immunodepression or a disorder involving immunodepression.

Thus, according to the present invention, there is provided the use of a compound of formula I,



I

wherein

R^1 and R^2 independently represent H or OH, or they may together represent a second carbon-carbon bond between

the carbon atoms to which they are attached;

R^3 represents methyl optionally substituted by $-CO_2H$ or an ester or amide thereof; ethyl optionally substituted by O, OH or $-CO_2H$ or an ester or amide thereof; propyl optionally substituted by OH or O; or allyl optionally substituted by OH;

R^4 represents H;

R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached;

R^7 represents H or OH;

R^8 represents OCH_3;

R^9 represents OH or OCH_3;

X represents O or (H,OH);

Y represents O or (H,OH); and

n represents 1 or 2;

in addition to their significances above

R^1 and R^5 may together represent an oxygen atom, in which case R^6 and R^7 together represent a second carbon-carbon bond between the carbon atoms to which they are attached;

R^7 and R^8 may together represent an oxygen atom; ar

R^3, R^4 and Y, together with the carbon atoms to which they are attached, may represent a methyl-substituted furanyl ring;

provided that

i) when R^2 represents H; R^3 represents methyl, ethyl,

propyl or allyl; R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached; R^8 represents OCH_3; and Y represents O; then R^7 represents OH; and

ii) when n is 1, then R^3 is not methyl or ethyl;

in the manufacture of a medicament for the treatment of immunodepression or a disorder involving immunodepression.

Examples of disorders involving immunodepression include AIDS, cancer, senile dementia, trauma (including wound healing, surgery and shock), chronic bacterial infection, and certain central nervous system disorders.

The immunodepression to be treated may be caused by an overdose of an immunosuppressive macrocyclic compound, for example derivatives of 12-(2-cyclohexyl-1-methylvinyl)-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene such as FR-900506, or rapamycin. Overdosing of such medicaments by patients is quite common upon their realising that they have forgotten to take their medication at the prescribed time, and can lead to serious side effects.

A further situation in which the compounds of formula I may be used to treat immunodepression is in vaccination. It is sometimes found that the antigen introduced into the body for the acquisition of immunity from disease acts as an immunosuppressive agent, and so antibodies are not produced by the body and immunity is not acquired. By introducing a compound of formula I into the body (for

example in the vaccine) the undesired immunosuppression may be overcome and immunity acquired.

The present invention further provides the novel compounds of formula I, as defined above, provided that

i) when R^1 represents OH; R^2 represents H; R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached; R^7 represents H; R^9 represents OCH_3; X and Y each represent O; and n represents 2; then R^3 does not represent 2-oxopropyl, 2,3-dihydroxypropyl or ethanalyl;

ii) when R^1 represents OH; R^2 represents H; R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached; R^7 represents OH; R^9 represents OCH_3; X and Y each represent O; and n represents 2; then R^3 does not represent allyl or 1-hydroxyprop-2-enyl; and

iii) when R^1 represents OH; R^2 represents H; R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached; R^7 represents H; R^9 represents OCH_3; X and Y each represent (H,OH); and n represents 2; then R^3 does not represent allyl.

In the novel use or the novel compounds, we prefer R^3 to be ethyl substituted by O or propyl substituted by O.

Desirably, R^7 is OH.

We prefer R^2 to be OH, more preferably (S)-OH (ie to have S absolute stereochemistry at its point of attachment

to the molecule).

When R^3 comprises an ester or amide group, we prefer the alcohol or amine moiety to contain from 1 to 10 carbon atoms, for example the alcohol moiety may be methanol.

Known compounds of formula I (as first defined above) from WO 89/05304 include:

17-Allyl-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-(1-Hydroxyprop-2-enyl)-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-(2,3-Dihydroxypropyl)-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Ethanalyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-(2-Oxopropyl)-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Allyl-1,2,14,16-tetrahydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-

13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-3,10-dione.

According to the invention there is further provided a process for the production of a novel compound of formula I, which comprises:

a) producing a compound of formula I, in which R^3 represents propyl substituted by O, by oxidation of a corresponding compound in which R^3 represents allyl;

b) producing a compound of formula I, which contains a vicinal diol, by oxidation of a carbon-carbon double bond in a corresponding compound;

c) producing a compound of formula I, in which R^3 represents ethyl substituted by O, by oxidative cleavage of a corresponding compound in which R^3 represents 2,3-dihydroxypropyl;

d) producing a compound of formula I, in which R^3 represents methyl substituted by $-CO_2H$ or ethyl substituted by $-CO_2H$, by oxidation of a corresponding compound in which R^3 represents ethanalyl or propanalyl;

e) producing a compound of formula I, which contains two vicinal hydrogen atoms, by reduction of a corresponding compound which contains a carbon-carbon double bond;

f) producing a compound of formula I, in which X or Y represents (H,OH), by reduction of a corresponding compound in which X or Y represents O;

g) producing a compound of formula I, in which R^3, R^4 and Y, together with the carbon atoms to which they are attached, represent a methyl-substituted furanyl ring, by

the action of acid on a corresponding compound in which R^3 represents 2-oxopropyl, R^4 represents H and Y represents O;

h) producing a compound of formula I, in which R^1 and R^5 together represent an oxygen atom and R^6 and R^7 together represent a second carbon-carbon bond between the carbon atoms to which they are attached, by the action of acid on a corresponding compound in which R^1 represents OH, R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached, and R^7 represents OH;

i) producing a compound of formula I, in which R^7 and R^8 together represent an oxygen atom, by the action of a dehydrating agent on a corresponding compound of formula I in which R^7 represents OH and R^8 represents OCH_3;

j) producing a compound of formula I, in which R^7 represents OH, by allylic oxidation of a corresponding compound in which R^7 represents H; or

k) producing a compound of formula I, in which R^3 represents allyl substituted by hydroxy, by allylic oxidation of a corresponding compound in which R^3 represents allyl.

Esters and amides of carboxylic acids that R^3 may represent may be produced by conventional methods.

Where desired or necessary, hydroxy groups may be protected and deprotected using conventional protecting group chemistry [as described in "Protective Groups in Organic Chemistry", ed: J W F McOmie, Plenum Press (1973),

and "Protective Groups in Organic Synthesis", T W Greene, Wiley-Interscience (1981)]. In addition, European patent application No 184162 describes the use of protecting groups in macrocyclic compounds.

In process (a), suitable oxidizing agents include a palladium (II) halide, for example palladium (II) chloride, in conjunction with a cuprous halide, for example copper (I) chloride. Suitable solvents include those that do not adversely affect the reaction, for example dimethylformamide (DMF) and water. The reaction is preferably carried out at a temperature of from 0 to 100°C, more preferably at or around room temperature.

In process (b), suitable oxidizing agents include osmium tetroxide, potassium permanganate, and iodine in conjunction with silver acetate. Osmium tetroxide is preferably used in conjunction with a regenerating agent such as 4-methylmorpholine N-oxide. Suitable solvents include those that do not adversely affect the reaction, for example diethyl ether or tetrahydrofuran (THF). In the case of potassium permanganate, aqueous alkaline conditions are preferred. The reaction is preferably carried out at a temperature of from 0 to 100°C, more preferably at or around room temperature.

In process (c), suitable reagents include lead tetraacetate and phenyliodoso acetate. Suitable solvents include those that do not adversely affect the reaction, for example benzene and glacial acetic acid. The reaction is preferably carried out at a temperature of from 0 to

100°C, more preferably at or around room temperature.

In process (d), suitable oxidizing agents include sodium chlorite in conjunction with sodium hydrogen phosphate. Suitable solvents include those that do not adversely affect the reaction, for example water. The reaction is preferably carried out at a temperature of from 0 to 100°C, more preferably at or around room temperature.

In process (e), the reduction may be carries out catalytically using hydrogen. Suitable catalysts include platinum catalysts (for example platinum black) and palladium catalysts (for example palladium-on-carbon). Suitable solvents include those that do not adversely affect the reaction, for example methanol and ethanol. The reaction may be carried out at or around room temperature.

In process (f), suitable reducing agents include borane (for example in the form of borane-ammonia complex) and sodium borohydride. Suitable solvents include those that do not adversely affect the reaction, for example diethyl ether and dichloromethane. The reaction may be carried out at or around room temperature. Where desired or necessary, the (H,OH) group may be oxidized back to O by the action of copper (II) acetate in acetic acid.

In processes (g) and (h), suitable acids include p-toluenesulphonic acid. Suitable solvents include those that do not adversely affect the reaction, for example toluene. The reaction is preferably carried out at a temperature above room temperature, for example on a steam bath.

In process (i), suitable reagents include Martin's sulphurane reagent. Suitable solvents include those that do not adversely affect the reaction, for example dichloromethane. The reaction is preferably carried out at a temperature below room temperature, for example -30°C.

In processes (j) and (k), suitable reagents include SeO_2, preferably in the presence of ^{t}butyl hydrogen peroxide. Suitable solvents include dichloromethane, and the reaction may be carried out at or around room temperature.

The invention further provides the use of the novel compounds of formula I as pharmaceuticals, and a pharmaceutical composition comprising such a compound in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

For the above-mentioned therapeutic uses the dosage administered will, of course, vary with the compound employed, the mode of administration, the treatment desired (eg topical, parenteral or oral) and the disease indicated. However, in general, satisfactory results are obtained when the compounds are administered at a dosage of from 0.1 to 200mg per kg of animal body weight.

For man the indicated total daily dosage is in the range of from 1mg to 100mg and preferably from 10mg to 500mg, which may be administered, for example twice weekly, or in divided doses from 1 to 6 times a day or in sustained release form. Thus unit dosage forms suitable for administration, eg oesophageally, comprise from 2mg to

500mg, and preferably 1mg to 500mg of the compound preferably admixed with a solid or liquid pharmaceutically acceptable diluent, carrier or adjuvant.

Suitable pharmaceutical compositions for administration of compounds of formula I (as first defined above) comprise (preferably less than 80%, and more preferably less than 50% by weight) of a compound of formula I (as first defined above) in combination with a pharmaceutically acceptable adjuvant, diluent or carrier. Examples of suitable adjuvants, diluents or carriers are: for tablets, capsules and dragees - microcrystalline cellulose, calcium phosphate, diatomaceous earth, a sugar such as lactose, dextrose or mannitol, talc, stearic acid, starch, sodium bicarbonate and/or gelatin; for suppositories - natural or hardened oils or waxes; and for inhalation compositions - coarse lactose. The compound of formula I (as first defined above) is preferably in a form having a mass median diameter of from 0.01 to 10 microns. The compositions may also contain suitable preserving, stabilising and wetting agents, solubilisers, sweetening and colouring agents and flavourings. The compositions may, if desired, be formulated in sustained release form. We prefer compositions which are designed to be taken oesophageally and to release their contents in the gastrointestinal tract.

We have also found that the toxicity of immunosuppressive compounds, particularly immunosuppressive macrocyclic compounds including derivatives of

12-(2-cyclohexyl-1-methylvinyl)-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene (for example FR-900506) and rapamycin, may be reduced by administering them in association with a compound of formula I as first defined above.

Thus, according to a second aspect of the invention, there is provided a pharmaceutical mixture comprising a compound of formula I (as first defined above) and an immunosuppressive compound.

Preferably, the greater proportion of active ingredient in such a mixture is the compound of formula I, for example the compound of formula I may be present at a ratio of greater than 10:1 by weight, for example 99:1.

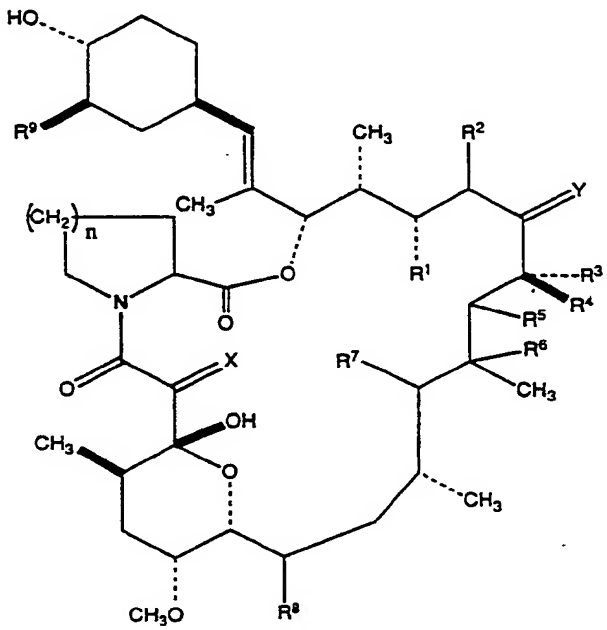
The invention also provides a method of treatment of immunodepression or a disorder involving immunodepression which comprises administering a therapeutically efficacious amount of a compound of formula I, as first defined above, to a patient suffering from such a condition.

The compounds of formula I (as first defined have the advantage that they are less toxic, more efficacious, are longer acting, have a broader range of activity, are more potent, produce fewer side effects, are more easily absorbed or have other useful pharmacological properties, than compounds previously used in the therapeutic fields mentioned above.

The compounds of formula I (as first defined above) have a number of chiral centres and may exist in a variety of stereoisomers. The invention provides the use of all

optical and stereoisomers, as well as racemic mixtures, and all optical and stereoisomers of the novel compounds of formula I per se.

However, the preferred stereochemistry of various chiral carbon atoms are shown in formula Ia,



Ia

wherein R^1 to R^8, X, Y and n are as defined above.

The invention is illustrated by the following examples.

Example 1

17-(2-Oxopropyl)-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

a) 17-(2-Oxopropyl)-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo

[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone

To a solution of palladium (II) chloride (25mg) and copper (I) chloride (50mg) in DMF (dimethylformamide) (6ml) and water (1ml) which had been previously oxygenated by having air bubbled through it for 30 minutes at room temperature was added a solution of 17-allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone (the compound of Example 4, WO 89/05304) (100mg) in DMF (2ml). The reaction mixture was then stirred and oxygenated for 3 hours at room temperature after which it was diluted with diethyl ether. The organic extract was then washed with dilute aqueous hydrochloric acid (1M) and brine before being dried ($MgSO_4$), filtered and evaporated to give the subtitle compound as an oil.

b) 17-(2-Oxopropyl)-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

The crude product of step (a) was then re-dissolved in dry methanol and was stirred with 10% Pd-on-carbon (10mg) under an atmosphere of hydrogen for 4 hours. The reaction mixture was then filtered, concentrated in vacuo and chromatographed on silica eluting with acetone/hexane [2:3] to give the title compound as a foam (84mg).

MS (FAB): 889 $[M+Rb]^+$; 827 $[M+Na]^+$; 805 $[M+H]^+$; 787 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 211.1 (C16); 207 (C41); 196.3 (C2); 169.1 (C10); 166.1 (C3); 138.7 (C19); 132 (C31); 131 (C29); 121.8 (C18); 97.3 (C1); 84 (C34); 82.4 (C12); 75.1 (C23); 56.2 (C9); 48.9 (C20); 47.6 (C17); 13.3 (C39)

Example 2

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid

A solution of FR-900506 (1.2g), 4-methylmorpholine N-oxide (1.3g) and osmium tetroxide (0.7ml of a 4% solution in water) in THF (tetrahydrofuran) (25ml) and water (14ml) was stirred for 3.5 hours at room temperature. Solid sodium metabisulphite was then added followed by ethyl acetate and Florisil (registered trade mark) and the combined mixture was filtered through celite. The separated organic extract (after washing with saturated aqueous sodium hydrogen carbonate solution) was dried ($MgSO_4$), filtered and evaporated in vacuo to give the crude 17-(2,3-dihydroxypropyl) compound as an oil. This was then dissolved in benzene (40ml) and lead tetraacetate (1.46g) was added. After stirring for 3 minutes at room temperature the reaction mixture was diluted with diethyl ether and was filtered, concentrated in vacuo, redissolved in diethyl ether, re-filtered and re-concentrated in vacuo to give the crude 17-(ethanalyl) compound as an oil. This was then dissolved in ^{t}butanol (25ml) and 1-methyl cyclohex-1-ene (6ml) and to this was added portionwise over

10 minutes solutions of sodium chlorite (0.8g) and sodium hydrogen phosphate (0.81g) each dissolved in 5ml of water. After stirring for 0.5 hours at room temperature the reaction mixture was quenched by the addition of ethyl acetate. Dilute aqueous hydrochloric acid (2M) was then added and the organic extracts (after washing with saturated aqueous sodium hydrogen carbonate solution) were dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with diethyl ether/methanol/acetic acid [190:9:1] and then further chromatography on silica eluting with dichloromethane/ methanol/acetic acid [170:9:1] gave the title compound as a foam (402mg, 32%).

MS (FAB): 906 $[M+Rb]^+$

^{13}C NMR ($CDCl_3$) δ: 196.3 (C2); 177.7(C41); 168.8 (C10); 164.6 (C3); 139.7 (C19); 132.7 (C29); 129.3 (C31); 120.6 (C18); 97.3 (C1); 84.2 (C34); 70.5 (C14); 9.8 (C39)

Example 3

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid Methyl ester

To a solution of the title compound of Example 2 (50mg) in dichloromethane (3ml) at room temperature was added a solution of diazomethane in diethyl ether until no starting material remained. The reaction mixture was then concentrated in vacuo and chromatographed on silica eluting with acetone/hexane [1:2] to give the title compound as a

foam (48mg).

MS (FAB): 920 $[M+Rb]^+$; 858 $[M+Na]^+$; 818 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 212.7 (C16); 196 (C2); 173 (C41); 168.8 (C10); 164.6 (C3); 139.5 (C19); 132.9 (C29); 129.1 (C31); 120.6 (C18); 97.2 (C1); 84.2 (C34); 70.8 (C14); 14.5 (C30); 9.7 (C39)

Example 4

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid N-Morpholine amide

A solution of the title compound of Example 2 (63.1mg), morpholine (20mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (28mg) and 4-dimethylaminopyridine (2mg) in dry dichloromethane (4ml) was stirred at room temperature for 2.5 hours. Water was then added and the reaction mixture was extracted with dichloromethane. After washing with dilute aqueous hydrochloric acid (1M) and saturated aqueous sodium hydrogen carbonate solution, the dichloromethane extracts were dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with hexane/acetone [1:1] then gave the title compound as a foam (58mg, 84%).

MS (FAB): 975 $[M+Rb]^+$; 891 $[M+H]^+$; 873 $[M-OH]^+$.

^{13}C NMR ($CDCl_3$) δ: 213.2 (C16); 195.9 (C2); 170.1 (C41); 168.8 (C10); 164.8 (C3); 139.1 (C19); 133.2 (C29); 128.9 (C31); 121.5 (C18); 97.4 (C1); 84.2 (C34); 66.3 and

65.8 (CO of morpholine); 10.1 (C39).

Example 5

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid N-Ethanolamide

A solution of the title compound of Example 2 (22.8mg) and 1,3-dicyclohexyl carbodiimide (6.7mg) in dry dichloromethane (2ml) was stirred at room temperature for 5 minutes. Ethanolamine (10mg) was then added and the reaction mixture was stirred at room temperature for 1.5 hours. Dilute aqueous hydrochloric acid (1M) was then added and the reaction mixture was extracted with dichloromethane. The organic extracts, after washing with saturated aqueous sodium hydrogen carbonate solution, were dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with dichloromethane/methanol/acetic acid [140:9:1] then gave the title compound as a glass (19.4mg, 81%).

MS (FAB): 950 $[M+Rb]^+$; 888 $[M+Na]^+$; 866 $[M+H]^+$; 848 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 198.7 (C2); 173.8 (C41); 169.2 (C10); 165.6 (C3); 140 (C19); 133.2 (C29); 129 (C31); 122.4 (C18); 97.8 (C1); 84.2 (C34); 61.2 (CO of ethanolamine); 44.0 (CN of ethanolamine); 16.2 (C47); 15.8 (C43); 14.7 (C30); 9.7 (C39)

Example 6

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-

methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid amide with glycine methyl ester

To a solution of the title compound of Example 2 (43.6mg) and methyl glycinate (3mg) in dichloromethane (3ml) was added triethylamine (22µl) and 2-chloro-1-methylpyridinium tosylate (24.2mg). After stirring at room temperature for 15 minutes dilute aqueous hydrochloric acid (2M) was added and the reaction mixture was extracted with dichloromethane. The organic extracts, after washing with saturated aqueous sodium hydrogen carbonate solution, were then dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with hexane/acetone [1:1] then gave the title compound as a foam (34.1mg, 72%).

MS (FAB): 977 $[M+Rb]^+$; 915 $[M+Na]^+$; 893 $[M+H]^+$; 875 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 213 (C16); 195.9 (C2); 171.7 (glycine carbonyl);170.3 (C41); 168.7 (C10); 164.7 (C3); 139.2 (C19); 132.9 (C29); 128.9 (C31); 120.9 (C18); 97.2 (C1); 84.1 (C34); 72.4 (C24); 71.1 (C14); 52.4 (OCH3 of glycine); 9.9 (C39)

Example 7

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid N-Piperidine amide

To a solution of 2-chloro-1-methylpyridinium tosylate (24.9mg) in dry dichloromethane (1ml) under nitrogen at room temperature was added a solution of the title compound of Example 2 (42.3mg), piperidine (10mg) and triethylamine (22μl) in dry dichloromethane (1.5ml). After 3 hours at room temperature a further portion of 2-chloro-1-methylpyridinium tosylate (15mg) and triethylamine (15μl) was added and stirring was continued for a further two hours at room temperature. Water was then added and the reaction mixture was extracted with dichloromethane. After washing with dilute aqueous hydrochloric acid (1M) and saturated aqueous sodium hydrogen carbonate solution, the dichloromethane extracts were dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with dichloromethane/methanol [29:1] then gave the title compound as a foam (13.3 mg, 29%).

MS (FAB): 973 $[M+Rb]^+$; 911 $[M+Na]^+$; 889 $[M+H]^+$

^{13}C NMR ($CDCl_3$) δ: 213.4 (C16); 196 (C2); 169.4 (C10); 168.6 (C41); 164.7 (C3); 138.8 (C19); 133.3 (C29); 128.7 (C31); 121.7 (C18); 97.3 (C1); 84.1 (C34); 10.1 (C39)

Example 8

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid N-Benzylamide

A solution of the title compound of Example 2 (48mg), 2-chloro-1-methylpyridinium tosylate (27.1mg),

triethylamine (25µl) and benzylamine (10mg) in THF (3ml) was stirred at room temperature for 1.5 hours. Dilute aqueous hydrochloric acid (2M) was then added and the mixture was extracted with ethyl acetate. The organic extracts, after washing with saturated aqueous sodium hydrogen carbonate solution, were dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with hexane/acetone [3:2] then gave the title compound as a foam (33.6 mg, 63%).

MS (FAB): 995 $[M+Rb]^+$; 933 $[M+Na]^+$; 911 $[M+H]^+$; 893 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 213.3 (C16); 196.1 (C2); 171.6 (C41); 168.9 (C10); 164.9 (C3); 139.3 (C19); 133.2 (C29); 128.9 (C_{ar}); 128 (C_{ar}); 121.4 (C18); 97.5 (C1); 84.3 (C34); 10.2 (C39)

Example 9

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid N-Butylamide

A solution of the title compound of Example 2 (57.8mg), 2-chloro-1-methylpyridinium tosylate (37.8mg), triethylamine (11.5µl) and butylamine (12mg) in THF (2ml) was stirred at room temperature for 3 hours. A further portion of 2-chloro-1-methylpyridinium tosylate (37.8mg) and triethylamine (11.5µl) were then added and stirring was continued for an additional 2 hours at room temperature, after which dilute aqueous hydrochloric acid

(2M) was added and the organic extracts (after washing with saturated aqueous sodium hydrogen carbonate solution) were dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with dichloromethane/methanol [29:1] then gave the title compound as a foam (36.3mg, 59%).

MS (FAB): 961 $[M+Rb]^+$; 899 $[M+Na]^+$; 877 $[M+H]^+$; 859 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 213.4 (C16); 196.1 (C2); 171.7 (C41); 168.9 (C10); 165 (C3); 139.2 (C19); 133.3 (C29); 129.2 (C31); 121.6 (C18); 97.5 (C1); 84.4 (C34); 10.2 (C39)

Example 10

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid p-Cyanophenyl ester

A solution of the title compound of Example 2 (51mg), 2-chloro-1-methylpyridinium tosylate (33.4mg), triethylamine (29μ) and p-cyanophenol (10.2mg) in THF (2ml) was stirred at room temperature for 2 hours. Dilute aqueous hydrochloric acid (2M) was then added and the organic extracts (after washing with saturated aqueous sodium hydrogen carbonate solution) were dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with dichloromethane/methanol [39:1] then gave the title compound as a foam (24.9 mg, 43%).

MS (FAB): 1007 $[M+Rb]^+$, 905 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 212.4 (C16), 196 (C2), 170.1

(C41), 168.8 (C10), 164.4 (C3), 153.8 (OC_{ar}), 140.4 (C19), 133.6 (C_{ar}), 132.7 (C29), 129.1 (C31), 122.6 (C_{ar}), 120.1 (C18), 118.2 (CN of p-cyanophenol), 109.7 ($C_{ar}CN$), 97 (C1), 84.1 (C34), 14.4 (C30), 9.9 (C39)

Example 11

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid Phenyl ester

A solution of the title compound of Example 2 (45mg), 2-chloro-1-methylpyridinium tosylate (32.3mg), triethylamine (30µl) and phenol (28mg) in dry dichloromethane (2ml) was stirred at room temperature for 2 hours. Dilute aqueous hydrochloric acid (1M) was then added and the organic extracts (after washing with saturated aqueous sodium hydrogen carbonate solution) were dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with hexane/ acetone [3:2] then gave the title compound as a foam (17.6mg, 36%).

MS (FAB); 982 $[M+Rb]^+$; 920 $[M+Na]^+$; 898 $[M+H]^+$; 880 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 212.4 (C16); 195.9 (C2); 171.1 (C41); 168.8 (C10); 164.5 (C3); 150.5 (OC_{ar}); 140 (C19); 132.7 (C29); 129.4 (C_{ar}); 129.3 (C31); 125.8 (C_{ar}); 121.4 (C_{ar}); 120.7 (C18); 97.2 (C1); 84.1 (C34); 9.7 (C39)

Example 12

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid p-Nitrophenyl ester

A solution of the title compound of Example 2 (150mg), 2-chloro-1-methylpyridinium tosylate (77mg), triethylamine (77µl) and p-nitrophenol (47mg) in dry dichloromethane (2ml) was stirred at room temperature for 2.5 hours. The reaction mixture was then concentrated in vacuo and purified directly by chromatography on silica eluting with hexane/diethyl ether [3:2] to give the title compound as a foam (135mg, 78%).

MS (FAB): 1027 $[M+Rb]^+$; 925 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 212.6 (C16); 196.2 (C2); 170.3 (C41); 169 (C10); 164.7 (C3); 155.4 (OC_{ar}); 145.5 (NC_{ar}); 140.6 (C19); 132.9 (C29); 129.3 (C31); 125.4 (C_{ar}); 122.6 (C_{ar}); 120.3 (C18); 97.2 (C1); 84.3 (C34); 9.8 (C39)

Example 13

17-Allyl-1,14,16-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10-trione

To a solution of FR-900506 (100mg) in diethyl ether/dichloromethane was added excess borane ammonia complex (150mg). After stirring for 2 hours at room temperature dilute aqueous hydrochloric acid (1M) was added and the ethereal extract was dried (Na_2SO_4), filtered

and evaporated in vacuo to give the crude 1,2,14,16-tetrahydroxy compound as an oil. This was taken up in acetic acid and copper (II) acetate (100mg) was added. After heating on a steam bath for 10 minutes the reaction mixture was cooled to room temperature and water was added. The reaction mixture was then extracted with ethyl acetate and the organic extracts, after washing with saturated aqueous sodium hydrogen carbonate solution, were dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with acetone/hexane [1:2] then gave the title compound (30mg) with R-stereochemistry at C16 followed by the title compound (24mg) with S-stereochemistry at C16.

(16R)-Stereoisomer

MS (FAB): 890 $[M+Rb]^+$

^{13}C NMR ($CDCl_3$) δ: 199.4 (C2); 169.1 (C10); 165.8 (C3); 136.9 (C41); 136.3 (C19); 132.6 (C29); 128.2 (C31); 125.4 (C18); 115.9 (C42); 98.7 (C1); 84.2 (C34); 56.5 (C9); 49 (C20); 43.2 (C17); 10.4 (C39)

(16S)-Stereoisomer

MS (FAB): 890 $[M+Rb]^+$; 828 $[M+Na]^+$; 806 $[M+H]^+$; 788 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 196.5 (C2); 169.3 (C10); 165.3 (C3); 137.8 (C41); 136.2 (C19); 132.9 (C29); 128.5 (C31); 126.6 (C18); 115.7 (C42); 97 (C1); 84.3 (C34); 56.4 (C9); 9 (C39)

Example 14

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-

methylvinyl]-26,28-dimethoxy-13,18,22,24,30-pentamethyl-11,17,31-trioxa-4-azatetracyclo[25.3.1.$0^{4,9}$.$0^{16,20}$] hentriaconta-16(20),18,21-triene-2,3,10-trione

A solution of 1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-(2-oxopropyl)-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone (the compound of Example 29, WO 89/05304) (100mg) in dry dichloromethane (25ml) containing p-toluenesulphonic acid (5mg) was heated under reflux for 30 minutes. Volatiles were then removed in vacuo and the residue was chromatographed on silica eluting with acetone/hexane [3:2] to give the title compound (19mg) as a foam.

MS (FAB): 887 $[M+Rb]^+$; 825 $[M+Na]^+$;803 $[M+H]^+$; 785 $[M-OH]^+$

^{13}C NMR ($CDCl_3$) δ: 195.9 (C2); 168.9 (C10); 164.9 (C3); 150.8 (C16); 148.3 (C18); 106.3 (C19); 97 (C1); 84.1 (C37); 8.5 (C42)

Example 15

1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propanalyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

A solution of FR-900506 (3g) in DMF (90ml) and water (15ml) containing palladium (II) chloride (0.4g) and copper (I) chloride (1.8g) was stirred at room temperature while air was bubbled through the reaction mixture for 2 hours. The reaction mixture was then diluted with diethyl ether and

the organic extract (after washing with dilute aqueous hydrochloric acid (1M), water and brine) was dried ($MgSO_4$), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with hexane in an increasing acetone gradient then gave the title compound as a foam (30mg).

MS (FAB): 821 [M+H]

^{13}C NMR ($CDCl_3$) δ: 213.1 (C16), 202.1 (C42), 196.3 (C2), 169 (C10), 164.7 (C3), 139.8 (C19), 132.6 (C29), 129.6 (C31), 121.9 (C18), 97.1 (C1), 84.2 (C34), 70.3 (C14), 9.6 (C39)

Example 16

17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28,29-trioxa-4-azatetracyclo[22.3.1.$1^{14,18}$.$0^{4,9}$] nonacos-19-ene-2,3,10,16-tetraone

A sample of 17-allyl-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone (as prepared in Example 13 of WO 89/05304, 60mg) was heated in toluene (10ml) containing p-toluenesulphonic acid for 10 minutes. Evaporation of the solvent in vacuo and chromatography on silica then gave the title compound as an oil (30mg).

^{13}C NMR ($CDCl_3$) δ: (single rotamer) 210.22 (C16); 195.96 (C2); 168.96 (C10); 165.24 (C3); 135.92 (C20); 134.69 (C41); 131.13 (C29); 128.78, 128.65 (C19, C31);

117.60 (C42); 97.53 (C1); 84.19 (C34); 80.79 (C18); 73.167 (C14); 52.47 (C17); 44.638 (C15); 29.992 (C21)

MS (FAB): 802.47 $[M+H]^+$; 886.2 $[M+Rb]^+$

Example 17

17-Propyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-25-dimethoxy-13,19,21,27-tetramethyl-11,28,29-trioxa-4-azatetracyclo[22.3.1.$1^{20,23}$,$0^{4,9}$]nonacos-18-ene-2,3,10,16-tetraone

A sample of 17-allyl-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone (as prepared in Example 13 of WO 89/05304, 121mg) was dissolved in dry dichloromethane (10ml) and to this was added Martin's sulphurane reagent at -30°C. After warming to 0°C, volatiles were removed in vacuo and the residue was chromatographed on silica to give a foam. To a solution of this in dry methanol (5ml) was added 10% Pd-on-carbon (10mg) and the resulting suspension was stirred in an atmosphere of hydrogen for 7 hours at room temperature. Volatiles were then removed in vacuo and the residue was purified by column chromatography on silica to yield the title compound as an oil (19mg).

MS (FAB): 770.76 $[M+H]^+$, 792.74 $[M+Na]^+$, 854.44 $[M+Rb]^+$

^{13}C NMR ($CDCl_3$) δ: (single rotamer) 197.81 (C16); 196.11 (C2); 168.96 (C10); 165.21 (C3); 146.16 (C14); 139.46 (C19); 135.92 (C41); 134.63 (C31); 129.51 (C29);

129.40 (C15); 125.31 (C18); 116.29 (C42); 97.33 (C1); 92.07 (C20); 84.09 (C34); 81.75 (C12); 80.03 (C23); 77.10 (C25); 74.67 (C24); 73.43 (C35); 56.78 (C9); 56.47 ($O\underline{C}H_3$); 55.95 ($O\underline{C}H_3$); 51.37 (C17)

Example 18

17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-25-methoxy-13,19,21,27-tetramethyl-11,28,29-trioxa-4-azatetracyclo[22.3.1.$1^{20,23}$.$0^{4,9}$]nonacosa-14,18-diene-2,3,10,16-tetraone

A sample of 17-allyl-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone (as prepared in Example 13 of WO 89/05304, 80mg) was dissolved in dry dichloromethane (5ml) and to this was added excess Martin's sulphurane reagent at -30°C portionwise until all the starting material had disappeared (30 minutes). The reaction mixture was then diluted with ethyl acetate and washed with water and then brine. The organic extract was dried ($MgSO_4$), filtered and evaporated in vacuo to an oil. Chromatography on silica then gave the title compound as an oil (20mg).

^{13}C NMR ($CDCl_3$) δ: (mixture of rotamers) 212.05, 211.04 (C16); 197.47, 194.95 (C2); 169.24, 169.09 (C10); 165.21, 164.72 (C3); 136.85, 137.44 (C19); 131.43, 130.97 (C29); 131.25, 128.90 (C31); 125.02, 124.35 (C18); 98.82, 97.44 (C1); 91.56, 91.29 (C20); 84.17 (C34)

MS (FAB): 775.11 $[M+H]^+$; 797.09 $[M+Na]^+$; 858.79

$[M+Rb]^+$

Example 19

12-[2-(4-Hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-1,14,15-trihydroxy-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$] octacos-18-ene-2,3,10,16-tetraone

A sample of 1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone as an oil (the compound of Example 11 of WO 89/05304, 510mg) was dissolved in dry tetrahydrofuran (12ml) and N-methyl morpholine N-oxide (0.3g) and osmium tetroxide (3ml of a 4% aqueous solution) were added. After stirring for 1 hour at room temperature solid sodium metabisulphite (1g) was added followed after 5 minutes by celite to produce a thick slurry. The reaction mixture was then diluted with ethyl acetate and filtered. The organic extract after washing with saturated aqueous sodium bicarbonate solution and brine was dried (magnesium sulphate), filtered and concentrated in vacuo to an oil. Column chromatogarphy on silica eluting with acetone/hexane [2:5] give two isomers of the title compound which differed in stereochemistry at C14 and/or C15.

Isomer 1 (180mg)

^{13}C NMR δ: (For the major rotamer) 211.82 (C16); 196.03 (C2); 169.10 (C10); 164.26 (C3); 139.28 (C19); 132.12 (C29); 128.22 (C31); 121.92 (C18); 95.65 (C1); 83.94 (C34); 75.76 (C12); 74.81 (C23); 74.63 (C15); 73.26 (C25);

73.26 (C35); 72.22 (C24); 71.66 (C14); 56.02 (C9); 48.05 (C20); 47.29 (C17); 39.08 (C5); 37.90 (C13); 34.92 (C33); 34.69 (C32); 34.47 (C27);33.66 (C22); 32.78 (C40); 32.63 (C26); 31.14 (C36); 30.57 (C37); 28.66 (C8); 25.78 (C21); 24.47 (C6); 21.24 (C7); 19.83 (C41); 19.83 (C44); 16.08 (C47); 15.66 (C43); 14.68 (C30); 14.07 (C42); 8.62 (C39)

^{1}H NMR δ: (For the Major rotamer) 5.48 (1H,brs,H-12); 5.15 (1H,d,J=9.6Hz,H-18); 5.08 (1H,d,J=8.8Hz,H-31); 3.79 (1H,dd,J=1.5 and 8Hz,H-24); 1.66 (3H,brs,H-30); 1.60 (3H,brs,H-43); 1.02 (3H,d,J=6.5Hz,H-47); 0.92 (3H,t,J=7.6 Hz,H-42); 0.90 (3H,d,J=7.6Hz,H-44); 0.83 (3H,d,J=7 Hz,H-39)

MS: 823 $[M+H]^+$; 845 $[M+Na]^+$; 906 $[M+Rb]^+$

Isomer 2 (32mg)

^{13}C NMR δ: (For the major rotamer) 212.99 (C16); 194.39 (C2); 169.60 (C10); 164.85 (C3); 138.33 (C19); 131.44 (C29); 129.95 (C31); 123.16 (C18); 97.79 (C1); 84.17 (C34); 76.54 (C12); 75.73 (C23); 75.68 (C15); 73.52 (C25); 73.52 (C35); 72.78 (C24); 71.88 (C14); 55.85 (C9); 49.21 (C17); 48.78 (C20); 39.36 (C5); 38.83 (C13); 36.04 (C40); 35.34 (C32); 34.83 (C33); 34.46 (C27); 33.21 (C22); 32.35 (C26); 31.21 (C36); 30.58 (C37); 28.34 (C8); 26.33 (C21); 24.75 (C6); 20.76 (C41); 20.63 (C7); 20.42 (C44); 16.31 (C47); 15.31 (C43); 14.17 (C30); 13.95 (C42); 9.64 (C39).

^{1}H NMR δ: (For the major rotamer) 5.24 (1H,brs,H-12); 4.74 (1H,t,J=3.4Hz,H-9); 3.66 (1H,dd,J=1.4 and 9.6Hz,H-24); 1.63 (3H,brs,H-30); 1.57 (3H,brs,H-43); 0.99 (3H,d,J=6.4Hz,H-47); 0.94 (3H,d,J=7.3Hz,H-39); 0.92 (3H,d,J=4.5Hz,H-44); 0.90 (3H,t,J=7.5 Hz,H-42)

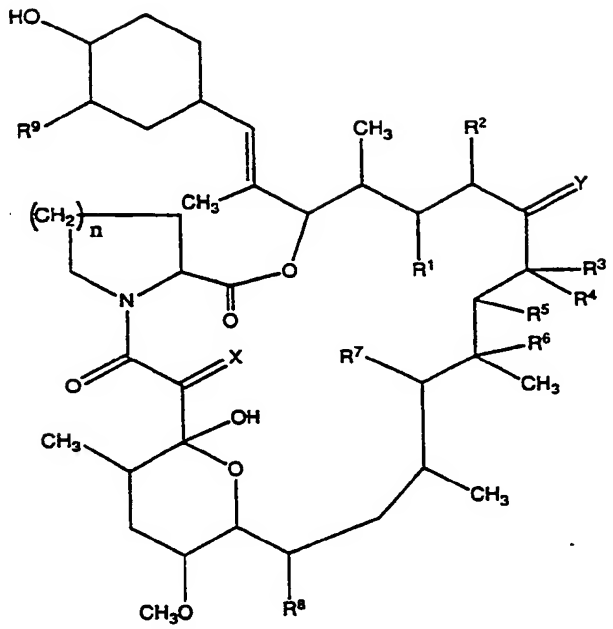
MS: 823 $[M+H]^+$; 845 $[M+Na]^+$; 906 $[M+Rb]^+$

Example 20

In the mixed lymphocyte reaction (MLR) (described in WO 89/05304, Example A), 17-(2-Oxopropyl)-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone, was found to have a pA_2 value of 8.3 with agonist compound FR-900506. This means that a concentration of $5x10^{-9}$M of the antagonist compound is required to occupy 50% of the available receptor sites in the screen for which it competes with FR-900506.

CLAIMS:

1. The use of a compound of formula I,



I

wherein

R^1 and R^2 independently represent H or OH, or they may together represent a second carbon-carbon bond between the carbon atoms to which they are attached;

R^3 represents methyl optionally substituted by $-CO_2H$ or an ester or amide thereof; ethyl optionally substituted by O, OH or $-CO_2H$ or an ester or amide thereof; propyl optionally substituted by OH or O; or allyl optionally substituted by OH;

R^4 represents H;

R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they

are attached;

R^7 represents H or OH;

R^8 represents OCH_3;

R^9 represents OH or OCH_3;

X represents O or (H,OH);

Y represents O or (H,OH); and

n represents 1 or 2;

in addition to their significances above

R^1 and R^5 may together represent an oxygen atom, in which case R^6 and R^7 together represent a second carbon-carbon bond between the carbon atoms to which they are attached;

R^7 and R^8 may together represent an oxygen atom; and

R^3, R^4 and Y, together with the carbon atoms to which they are attached, may represent a methyl-substituted furanyl ring;

provided that

i) when R^2 represents H; R^3 represents methyl, ethyl, propyl or allyl; R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached; R^8 represents OCH_3; and Y represents O; then R^7 represents OH; and

ii) when n is 1, then R^3 is not methyl or ethyl;

in the manufacture of a medicament for the treatment of immunodepression or a disorder involving immunodepression.

2. A compound of formula I, as defined in claim 1,

provided that

i) when R^1 represents OH; R^2 represents H; R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached; R^7 represents H; R^9 represents OCH_3; X and Y each represent O; and n represents 2; then R^3 does not represent 2-oxopropyl, 2,3-dihydroxypropyl or ethanalyl;

ii) when R^1 represents OH; R^2 represents H; R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached; R^7 represents OH; R^9 represents OCH_3; X and Y each represent O; and n represents 2; then R^3 does not represent allyl or 1-hydroxyprop-2-enyl; and

iii) when R^1 represents OH; R^2 represents H; R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached; R^7 represents H; R^9 represents OCH_3; X and Y each represent (H,OH); and n represents 2; then R^3 does not represent allyl.

3. The use of a compound of formula I, as defined in claim 1, or a compound of formula I as defined in claim 2, wherein R^3 is ethyl substituted by O or propyl substituted by O.

4. The use of a compound of formula I, as defined in claim 1, or a compound of formula I as defined in claim 2, wherein R^7 is OH.

5. The use of a compound of formula I, as defined in claim 1, or a compound of formula I as defined in claim 2,

wherein R^2 is OH.

6. The use of a compound of formula I, as defined in claim 1, wherein the compound of formula I is:

17-Allyl-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-(1-Hydroxyprop-2-enyl)-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-(2,3-Dihydroxypropyl)-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Ethanalyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-(2-Oxopropyl)-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone, or

17-Allyl-1,2,14,16-tetrahydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-3,10-dione.

7. A compound of formula I, as defined in claim 2, which

is:

17-(2-Oxopropyl)-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone,

17-(2-Oxopropyl)-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid,

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid Methyl ester,

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid N-Morpholine amide,

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl}-ethanoic acid N-Ethanolamide,

2-{1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-

2,3,10,16-tetraone-17-yl)-ethanoic acid amide with glycine methyl ester,

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid N-Piperidine amide,

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid N-Benzylamide,

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid N-Butylamide,

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid p-Cyanophenyl ester,

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid Phenyl ester,

2-(1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone-17-yl)-ethanoic acid p-Nitrophenyl ester,

17-Allyl-1,14,16-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10-trione

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-26,28-dimethoxy-13,18,22,24,30-pentamethyl-11,17,31-trioxa-4-azatetracyclo[25.3.1.$0^{4,9}$.$0^{16,20}$] hentriaconta-16(20),18,21-triene-2,3,10-trione,

1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propanalyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28,29-trioxa-4-azatetracyclo [22.3.1.$1^{14,18}$.$0^{4,9}$]nonacos-19-ene-2,3,10,16-tetraone,

17-Propyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-25-dimethoxy-13,19,21,27-tetramethyl-11,28,29-trioxa-4-azatetracyclo[22.3.1.$1^{20,23}$.$0^{4,9}$]nonacos-18-ene-2,3,10,16-tetraone,

17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-25-methoxy-13,19,21,27-tetramethyl-11,28,29-trioxa-4-azatetracyclo[22.3.1.$1^{20,23}$.$0^{4,9}$]nonacosa-14,18-diene-2,3,10,16-tetraone, or

12-[2-(4-Hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-1,14,15-trihydroxy-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$] octacos-18-ene-2,3,10,16-tetraone.

8. The use of a compound of formula I, as defined in claim 2, as a pharmaceutical.

9. A pharmaceutical composition comprising a compound of formula I, as defined in claim 2, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

10. A pharmaceutical mixture comprising a compound of formula I as defined in claim 1 or claim 2, and an immunosuppressive compound.

11. A process for the production of a compound of formula I, as defined in claim 2, which comprises:

a) producing a compound of formula I, in which R^3 represents propyl substituted by O, by oxidation of a corresponding compound in which R^3 represents allyl;

b) producing a compound of formula I, which contains a vicinal diol, by oxidation of a carbon-carbon double bond in a corresponding compound;

c) producing a compound of formula I, in which R^3 represents ethyl substituted by O, by oxidative cleavage of a corresponding compound in which R^3 represents 2,3-dihydroxypropyl;

d) producing a compound of formula I, in which R^3 represents methyl substituted by $-CO_2H$ or ethyl substituted by $-CO_2H$, by oxidation of a corresponding compound in which R^3 represents ethanalyl or propanalyl;

e) producing a compound of formula I, which contains two vicinal hydrogen atoms, by reduction of a corresponding compound which contains a carbon-carbon double bond;

f) producing a compound of formula I, in which X or Y

represents (H,OH), by reduction of a corresponding compound in which X or Y represents O;

g) producing a compound of formula I, in which R^3, R^4 and Y, together with the carbon atoms to which they are attached, represent a methyl-substituted furanyl ring, by the action of acid on a corresponding compound in which R^3 represents 2-oxopropyl, R^4 represents H and Y represents O;

h) producing a compound of formula I, in which R^1 and R^5 together represent an oxygen atom and R^6 and R^7 together represent a second carbon-carbon bond between the carbon atoms to which they are attached, by the action of acid on a corresponding compound in which R^1 represents OH, R^5 and R^6 together represent a second carbon-carbon bond between the carbon atoms to which they are attached, and R^7 represents OH;

i) producing a compound of formula I, in which R^7 and R^8 together represent an oxygen atom, by the action of a dehydrating agent on a corresponding compound of formula I in which R^7 represents OH and R^8 represents OCH_3;

j) producing a compound of formula I, in which R^7 represents OH, by allylic oxidation of a corresponding compound in which R^7 represents H; or

k) producing a compound of formula I, in which R^3 represents allyl substituted by hydroxy, by allylic oxidation of a corresponding compound in which R^3 represents allyl.

12. A method of treatment of immunodepression, or a

disorder involving immunodepression, which comprises administering a therapeutically efficacious amount of a compound of formula I, as defined in claim 1, to a patient suffering from such a condition.

4002complete/jrh

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01412

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 31/33, C 07 D 498/18, 498/22//(C 07 D 498/18, 311:00 273:00, 221:00),(C 07 D 498/22, 311:00, 311:00, 273:00, 221:00)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 07 D; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP, A3, 0184162 (FUJISAWA PHARMACEUTICAL CO., LTD.) 11 June 1986, see the whole document	2,7,9-11
X	WO, A1, 8905304 (FISONS PLC) 15 June 1989, see the whole document	2,7,9-11
P,X	EP, A2, 0349049 (MERCK & CO. INC.) 3 January 1990, see the whole document	2,7,9-11
P,X	EP, A2, 0349061 (MERCK & CO. INC.) 3 January 1990, see the whole document	2,7,9-11

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19th December 1990	17. 01. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. Peis (M. PEIS)

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	EP, A2, 0356399 (SANDOZ AG) 28 February 1990, see the whole document	2,7,9-11

--

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 8 and 12, because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods /see PCT Rule 39(i)/.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional seach fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 90/01412

SA 40366

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/11/90 The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A3- 0184162	11/06/86	AU-B-	592067	04/01/90
		AU-D-	5059685	12/06/86
		JP-A-	61148181	05/07/86
		US-A-	4894366	16/01/90
		US-A-	4929611	29/05/90
		US-A-	4956352	11/09/90
WO-A1- 8905304	15/06/89	AU-D-	2822889	05/07/89
		EP-A-	0323042	05/07/89
		EP-A-	0346427	20/12/89
EP-A2- 0349049	03/01/90	NONE		
EP-A2- 0349061	03/01/90	JP-A-	2188585	24/07/90
EP-A2- 0356399	28/02/90	AU-D-	4024689	01/03/90
		JP-A-	2167287	27/06/90

For more details about this annex : see Official Journal of the European patent Office, No. 12/82

EPO FORM P0479